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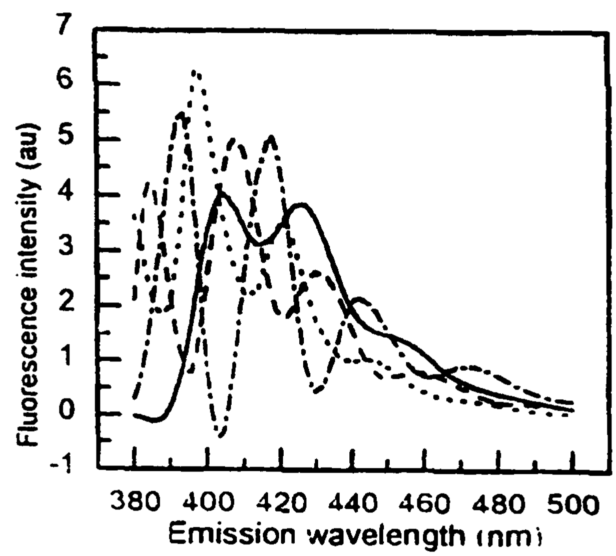
(54) Title: METHOD FOR CHARACTERIZING SAMPLES

(57) Abstract

The present invention relates to a method for characterizing single test samples using techniques generating multi dimensional responses from which the components of the sample can be identified. The method does not require any references and is applicable even on samples which are less than the number of components they contain.



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METHOD FOR CHARACTERIZING SAMPLES

The present invention relates to methods for characterizing samples. These methods are i.a. used to investigate test samples from a production, patients or samples collected in any other way.

Background of the invention

When a sample is to be characterized for components, the components are generally separated from each other in a first step in order to identified and quantified in a later stage. However, it is not always possible to separate the components or it may not be motivated from a time/cost benefit reason. The samples may then be characterized spectroscopically whereby the components are identified by means of their unique spectral responses.

If one has a collection of samples and is aware of which components they comprise, it is, as a rule, trivial to determine their concentrations spectroscopically. This is due even if the spectral responses of the components overlaps each other. If, however, the components are unknown, the problem is muck more complicated . The situation was analysed for the first time in detail by the mathematics Lawton and Sylvestre (Technometrics, **13**, 617, (1971)), who showed that it is impossible to find an unique solution even for a 2-component system. In 1990 we developed an experimental method, which partly solved this problem (Kubista, Chemometrics and Intelligent Laboratory Systems, **7**, 273, (1990)). We then showed that if one carried out two spectroscopic measurements on each sample, in stead of one as previously used, and the measurements were such that the contribution of the components to these measurements had the same distribution of the intensities, but of different magnitude, then both the spectral responses as well as the concentrations of the components could be determined. Mathematically, these measurements are described using the equations:

$$\mathbf{A} = \mathbf{CV} \text{ or } a_j(\lambda) = \sum_{i=1}^{r} c_{ij} v_i(\lambda) \qquad j = 1,2 \ldots n$$

$$\mathbf{B} = \mathbf{CDV} \text{ or } b_j(\lambda) = \sum_{i=1}^{r} c_{ij} d_j v_i(\lambda) \qquad j = 1,2 \ldots n$$

wherein **A** is a matrix comprising spectra of the first type measured on the **n** samples; **B** is a matrix comprising spectra of the second type measured on the same **n** samples; **C** is a

matrix comprising the concentrations of the **r** different components in the **n** samples; **V** is a matrix comprising the normalized spectra of the components; and **D** is a diagonal matrix, the **r** diagonal elements of which being the ratios between the responses of the components obtained in the two measurements. All spectra are digitalized in **m** points. We showed that the concentrations of the components (**C**), their normalized spectral responses (**V**) and the ratio between their responses obtained in the two measurements (**D**) could be determined only outgoing from the information obtained from the spectra as measured (**A** and **B**). We further described how the number of components of the samples (**r**) could be estimated.

One restriction using this method is that the number of components are not allowed to exceed the number of samples, which from a practical point of view means that the method can not be utilized on smaller series of samples and can not be applied on the whole for analysing isolated samples.

Several spectroscopic techniques, such as fluorescence, nmr, etc., can generate 2-dimensional data described by the equation:

$$I(\alpha,\beta) = \kappa\sum_{i=1}^{r} I_i(\alpha)c_i I_i(\beta)$$

where the signal, $I(\alpha,\beta)$, is determined as a function of two variables, α and β, and are the sum of the contribution of the components in each point, which contribution is proportional to their concentrations (c_i) and the products of their (normalized) 1-dimensional responses, $I_i(\alpha)$ and $I_i(\beta)$. Out of these responses the components can be identified. In a steady state fluorescence spectroscopy $I_i(\alpha)$ and $I_i(\beta)$ are the excitation- and emissions spectra of the components and are, as a rule, designated $I_i^{ex}(\lambda_{ex})$ and $I_i^{em}(\lambda_{em})$, wherein λ_{ex} and λ_{em} are the excitation and emission wavelengths. The shape of an excitation spectra of a pure compound is, in general independent of the emission wavelength used at the measurement, and the corresponding is due for its emission spectrum. The fluorescence signal monitored, if necessary after a correction for the inner filter effect (Kubista et al, The Analyst, **119**, 417 (1994)), is proportional to the concentration of the compound. In a sample containing more compounds the total signal is the sum of the contribution by each component. As fluorescence is measured in an arbitrary unit, eq. 1 contains a proportionality constant (κ).

The information of the 2-dimensional spectrum $I(\alpha,\beta)$ is insufficient to unambiguously

determine the spectral responses of the components. Different approximative ways have been suggested but these do not function sufficient satisfactorily even for a 2-component mixture (Burdick and Tu, J. Chemometrics, **3**, 431, (1989)).

The present invention is a method for analysing isolated test samples, or a couple of test samples without using references in such a way that the components can be identified.

Description of the figures.

Figure 1. Emission spectra monitored using different excitation wavelengths using a parallel polarized light (above, left) and a perpendicularly polarized light (above right), respectively. Down to the left the calculated emission spectra of the components are shown, and down to the right the calculated excitation spectra of the components are shown.

Figure 2. A) Excitation spectra registered using different emission wavelengths from two solutions containing POPOP, dimethyl POPOP, antracene, and diphenyl antracene. B) The excitation spectra of the components as calculated.

Figure 3. A) Emission spectra registered using different excitation wavelengths of two solutions containing POPOP, dimethyl POPOP, antracene, and diphenyl antracene. B) The excitation spectra of the components as calculated.

Brief description of the invention

The present invention is a method for analyzing test samples in such a way that its components can be identified without the need for any reference data. The method is based upon the following four steps:

1. The test sample is analyzed using a method generating a 3-dimensional response according to :

$$I(\alpha,\beta,\gamma)=\sum_{i=1}^{r}\tilde{I}_i(\alpha)\tilde{I}_i(\beta)\tilde{I}_i(\gamma),$$

wherein **r** is the number of components contributing to the signal, and $\tilde{I}_i(\alpha)$ and $\tilde{I}_i(\beta)$ and $\tilde{I}_i(\gamma)$ are the arbitrarily normalized 1-dimensional responses of the components, which responses normally consist of spectral or concentration variations.

2. The number of components **r** as the samples contain is estimated.

3. For each component its 1-dimensional responses $I_i(\alpha)$ and $I_i(\beta)$ and $I_i(\gamma)$ are determined.

4. Out of the responses, the components are identified.

Detailed description of the present invention

As the title indicates the present invention relates to a method for characterizing isolated test samples in a way that makes it possible to identify its components without any need for using reference samples. This is done through a strategic design of experiments which makes it possible to register a 3-dimensional response being proportional to the concentrations of the components, and the contribution from each component is the product of its specific 1-dimensional responses:

$$I(\alpha,\beta,\gamma)=\sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

Such registration can be carried using certain forms of fluorescence spectroscopy, e.g., by means of a time disintegrated monitoring of emission/excitation spectra, i.e., the signal is registered as a function of excitation wavelength, emission wavelength, and time:

$$I(\lambda_{ex},\lambda_{em},t)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(t)$$

In these cases it is often suitable to gather the concentration of the components ci and the time declinations to a time dependent concentration:

$$I(\lambda_{ex},\lambda_{em},t)=\sum_{i=1}^{r} c_i(t) I_i(\lambda_{ex}) I_i(\lambda_{em})$$

The time can be time after light pulse (whereby $c_i(t)$ is proportional to the fluorescence declination), time after mixing of e.g., a stop-flow experiment (whereby $c_i(t)$ is the variation of the concentration of component *i* with time), time after treatment, such a photo bleaching (selective destruction of certain components using light), chromatographic or other form of separation, etc. At the analysis of such data the concentration variation of the components are calculated, as well as their excitation and emission spectra. It is of interest to note that intermediate components which are neither present at the beginning ($c_i(0) = 0$) or at the end ($c_i(\infty) = 0$) of the experiment can be identified from its calculated spectra.

There is a further possibility in varying the polarization of the light:

$$I(\lambda_{ex},\lambda_{em},\alpha)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

or, if the phase-modulated light is utilized, the frequency of the modulation:

$$I(\lambda_{ex},\lambda_{em},\nu)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\nu)$$

etc.

There is further a possibility in varying the outer parameters which influences the concentrations of the components, such as temperature (pressure, volume, etc.):

$$I(\lambda_{ex},\lambda_{em},T)=\sum_{i=1}^{r}c_i(T)I_i(\lambda_{ex})I_i(\lambda_{em})$$

or outer parameters which influence the intensity of the responses of the components, such as external magnetic fields (electrical fields, etc.):

$$I(\lambda_{ex},\lambda_{em},M)=\sum_{i=1}^{r}c_iI_i(\lambda_{ex})I_i(\lambda_{em})I_i(M).$$

The spectroscopic technique need not be a fluorescence technique. The method can be carried out using most techniques which generates 3-dimensional responses, e.g., nuclear magnetic resonance spectrometry (NMR) mass spectrometry, etc. It can further be carried out using most techniques generating 2-dimensional responses if the responses of the components influence external parameters. Finally, the method can be used using a technique generating 1-dimensional responses, as well, but then it is necessary that two external parameters are varied simultaneously and that their influence on the responses of the components are independent so that their contribution can be factorized.

The invention requests that at least two data points are determined in each of the 3 dimensions, i.e.:

$I_i(\alpha)$ wherein $\alpha_1,\alpha_2.....\alpha_n$ $1\geq2$
$I_i(\beta)$ wherein $\beta_1,\beta_2.....\beta_m$ $m\geq2$
$I_i(\gamma)$ wherein $\gamma_1,\gamma_2.....\gamma_l$ $n\geq2$

To determine two data points only in all dimensions are, however, of particular meaning as the tolerance of the responses calculated then as a rule is insufficient to be able to identify the components. On the contrary it is quite excellent to have two data points only in one of the dimensions , e.g, 1=2 (and m>>2, and n>>2). This exhibits the advantage that the numerical treatment of data is made easier as the responses of the components can be calculated using fast algorithms such as Procrustes rotation and GRAM (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990); Wilson, Sanches & Kowalski, J. Chemometrics, 3, 493, (1989)). In the general case when all l, n, and m are greater than 2, the

solution method is much more complicated and thus considerably more time consuming (Liwo, et al, Computers Chem., **21**, 89-91, (1997)). Furthermore, it is quite often of interest to carry out the experiment in such a way that one of m and n are considerably greater than the other. The reason hereto is that it as a rule, is sufficient for the identification of the components, to determine one of their 1-dimensional responses with a high accuracy.

The invention is not limited to determinations that generates 3-dimensional responses but even responses of a higher order can be used. In general it should be satisfying that the response is linear and that the contribution from each component shall be the product of its 1-dimensional responses:

$$I(\alpha,\beta,\gamma,\delta....) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta)....$$

Of course, the higher the dimension is the more time consuming the numerical treatment of the determined data will become. However, with regard to the very fast development within the computer area this will hardly be a practical limitation in the future.

The samples to be analysed shall contain substantially the same components, and these shall be present in different, relative concentrations. The samples are analysed in pair using a 2-dimensional method which provides a response which is proportional to the concentrations of the components and the product of the 1-dimensional responses. This can be expressed as:

$$I^A(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^A I_i(\beta)$$

$$I^B(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^B I_i(\beta)$$

wherein $I^A(\alpha)$ and $I^B(\beta)$ are spectra of the two samples which in the following will be called A and B, determined as a function of the variables α and β, r is the total number of components contributing to the spectra, $I_i(\alpha)$ and $I_i(\beta)$ are the normalized 1-dimensional responses of the components, and c_i^A and c_i^B are their concentrations, respectively. In a steady-state fluorescence spectroscopy $I_i(\alpha)$ are the normalized excitation spectra of the components, *$I_i^{ex}(\lambda_{ex})$, and $I_i(\beta)$ are their normalized emission spectra, $I_i^{em}(\lambda_{em})$:*

$$I^A(\lambda_{ex},\lambda_{em}) = \sum_{i=1}^{r} I_i^{ex}(\lambda_{ex}) c_i^A I_i^{em}(\lambda_{em})$$

$$I^B(\lambda_{ex},\lambda_{em}) = \sum_{i=1}^{r} I_i^{ex}(\lambda_{ex}) c_i^B I_i^{em}(\lambda_{em})$$

The information in these spectra is treated in two steps. First the number of components, r, is determined, and then the 1-dimensional responses of the components, $I_i^{ex}(\lambda_{ex})$, and $I_i^{em}(\lambda_{em})$.

When ***r*** has been determined, the spectral responses of the components.
The equations:

$$I^A(\lambda_{ex},\lambda_{em}) = \sum_{i=1}^{r} I_i^{ex}(\lambda_{ex}) c_i^A I_i^{em}(\lambda_{em})$$

$$I^B(\lambda_{ex},\lambda_{em}) = \sum_{i=1}^{r} I_i^{ex}(\lambda_{ex}) c_i^B I_i^{em}(\lambda_{em})$$

can be written in matrix form as:

$\mathbf{A=XC^AM}$

$\mathbf{B=XC^BM}$

wherein **A** and **B** are matrixes comprising the spectra determined, **X** is a matrix comprising the normalized excitation spectra of the components, **M** is a matrix comprising their normalized emission spectra, and $\mathbf{C^A}$ and $\mathbf{C^B}$ are diagonal matrixes comprising the concentrations of the components. By renormalizing one of **X** or **M**, the equation system can be rewritten as:

$\mathbf{A=XM}$

$\mathbf{B=XDM}$

wherein **D** is a diagonal matrix comprising the ratios between the concentrations of the components ($\mathbf{D=C^B/C^A}$). Using **A** and **B, X, M** and **D** can be calculated using known methods such as Procrustes rotation (Kubista, <u>Chemometrics and Intelligent Laboratory Systems</u>, 7, 273, (1990); and GRAM (Wilson, Sanches & Kowalski, <u>J. Chemometrics</u>, 3, 493, (1989).

As a summary, the present invention relates to a method for experimentally studying two samples spectroscopically so that the information present in the experimental spectra is sufficient to determine the number of components of the samples (r), their spectral responses of the 1st dimension, $I_i(\alpha)$, their spectral responses of the 2nd dimension, $I_i(\beta)$, and the ratios between their concentrations ($\mathbf{c_i^A/c_i^B}$).

The most apparent use of the invention is for the analysis of two samples containing common components. All components need not be common, but the majority of those contributing spectroscopically should be in common (Booksh & Kowalski, J. Chemotrics, 8, 287, (1994)). The number of components is arbitrary and can exceed 2.

Another use of the invention is to characterize single samples by first dividing them into two part samples containing the ingoing components in different proportions. This can be accomplished in several ways, e.g., by filtering, extracting, chromatographying dialysing, centrifuging, precipitating, splitting the sample by means of an electrical field, etc. Alternatively, the original sample can be used as one sample, and an aliquot thereof, which is created in such a way that the components are present in other proportions, is used as the second sample. This aliquot can be obtained by selectively eliminating certain components, e.g., by means of adsorption, precipitation, freezing, distillation, selective decomposing (e.g., by light, heat, radio lysis), etc. Another possibility is to create two samples from one, is to change the conditions for the determination, e.g., by changing the temperature, pressure, etc. Separation methods, such as different types of chromatography are of interest, as the components are separated in space, and one, principally arbitrary number of samples can be obtained which can be analysed in pair. Using spectroscopic techniques which generates 2-dimensional spectra in a fast way, then, furthermore, the detection can be made on-line.

Another use of the invention is to determine the concentrations of the components in one test sample in relation to a standard sample with a high degree of accuracy. The standard sample and the test sample are analysed as a pair, and the ratio between the concentrations of the components is obtained as the diagonal element of the **D** matrix.

2-dimensional spectra wherein one of the dimensions is time, are of particular interest, whereby time is related to time after a disturbance such as a relaxation time. Today, there are e.g., fluorescence instruments by means of which one can determine complete spectra as a function of time after lightening (either directly after lightening using a light pulse, or indirectly using phase modulation technique). This gives using α as time, and β as wave length, the equation system:

$$I^A(t,\lambda) = \sum_{i=1}^{r} I_i(t) c_i^A I_i(\lambda)$$

$$I^B(t,\lambda) = \sum_{i=1}^{r} I_i(t) c_i^B I_i(\lambda)$$

from which r, $I_i(t)$, $I_i(\lambda)$ and (c_i^A/c_i^B) can be determined.

Example

The invention will be further illustrated in four examples.

Example 1

A sample is characterized using fluorescence spectroscopy, where excitation wave length, emission wave length, and light polarization are varied (Figure 1). This gives raise to a 3-dimensional spectrum according to:

$$I(\lambda_{ex}, \lambda_{em}, \alpha) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

In the example 650 different emission wave lengths (m), 11 different excitation wave lengths (n) and 2 different polarizations (α = 0o, called parallel polarization, and α = 90o, called perpendicular polarization) (l), are used. From the response determined, $In(\lambda_{ex}, \lambda_{em}, \alpha)$, first the number of components (r) is estimated to 2 (using a statistic test and a visual inspection of the principal components). Then the component specific responses are calculated. For this purpose one uses the fact that only two data points were registered in one of the dimensions (polarization) and rewrote the 3-dimensional response to two 2-dimensional responses.

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 0°) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 0°)$$

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 90°) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 90°)$$

These can be described using the equation system:

$\mathbf{I^0 = X\alpha^0 M}$

$\mathbf{I^{90} = X\alpha^{90} M}$

which can be solved using Procrustes rotation (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, (1990)). This gave the normalized excitation intensities of the components as matrix X($I_i(\lambda_{ex})$), (shown down to the right in Figure 1), the normalized emission intensities of the components as matrix M ($I_i(\lambda_{em})$) (shown down to the left in Figure 1), and the ratios between the responses of components to light of different polarization

From the calculated component specific responses, in particular the emission spectra, the component could be identified as p-bis[2-(5-phenyloxazolyl)]-benzene (POPOP), and antracene. Finally, by comparing standard spectra of POPOP and antracene the concentrations could be estimated to some micro molars.

Example 2

Two solutions containing the dye compounds POPOP, dimethyl POPOP, antracene and diphenyl antracene in different proportions were prepared. On these fluorescence excitation spectra were monitored at several emission wave lengths. The number of components were determined to 4 using a statistic test, and the excitation spectra of the components (Figure 1), emission intensities and the relation between their concentrations in the two samples were calculated.

Example 3

On the same solutions as in Example 1 the fluorescence emission spectra were monitored using a number of excitation wave lengths . The number of components was determined to 4 using a statistic test, and the emission spectra of the components (Figure 2), excitation intensities and the relation between their concentrations in the two samples were determined.

Example 4

Characterization of samples containing the dye compound thiazole orange and the polymer poly(dG) was made. The samples were analysed in pairs using 2-dimensional fluorescence spectroscopy. They contains thiazole orange and poly(dG) in the relation [thiazole orange]/[poly(dG)] of 0.05 and 0.025. Neither poly(dG) nor the dye compound is fluorescentic as such but the fluorescence arises when thiazole orange binds to the polymer. The samples were analysed in two different ways. In one analysis, the fluorescence excitation spectra were monitored at different emission wave lengths. The number of fluorescent components were identified to two using statistic tests, and their excitation spectra and emission intensities were calculated.. In the second analysis, the fluorescence emission spectra were monitored using a number of excitation wave lengths. Once again the number of components was identified to two, and their emission spectra and excitation intensities were calculated.

CLAIMS

1. A method for characterizing a sample,

characterized in that

a) a sample, or pair of samples, is (are) characterized using a monitoring technique such that a multi dimensional response is generated according to

$$I(\alpha,\beta,\gamma,\delta....) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta)....,$$

b) the response monitored ir broken down to an orthogonal basset e.g., using a principal component division,

c) the number of components (r) in the sample is estimated,

d) the arbitrary normalized 1-dimensional responses of the components are calculated.

2. A method according to claim 1, wherein the number of samples is two and these are analysed using a method generating a 2-dimensional response according to

$$I(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i I_i(\beta)$$

and the 1-dimensional responses of the components and the ratios between their concentrations in the two samples, (c_i^A/c_i^B), is calculated by solving the equation system

$$I^A(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^A I_i(\beta)$$

$$I^B(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^B I_i(\beta)$$

3. A method according to claim 2, wherein the two samples are generated from one sample.

4. A method according to claim 1 or 2, wherein one of the samples is used as a standard sample to determine the concentrations of the components in a test sample.

5. A method according to claim 1, wherein a single sample is amalysed using a technique generating 3-dimensional response:

$$I(\alpha,\beta,\gamma) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

and the arbitrary normalized 1-dimensional responses of the components, $\widetilde{I}_i(\alpha)$ and $\widetilde{I}_i(\beta)$ and $\widetilde{I}_i(\gamma)$ are calculated.

6. A method according to claim 1, wherein a single sample is analysed using a technique generating a 2-dimensional response simultaneously as external parameters are varied in such a way that the concentration of the components are changed in time:

$$I(\alpha,\beta,\gamma)=\sum_{i=1}^{r}c_i(t)I_i(\alpha)I_i(\beta)$$

and the arbitrary normalized 1-dimensional responses, $\widetilde{I}_i(\alpha)$ and $\widetilde{I}_i(\beta)$ and their changes as to concentration $c_i(t)$ is calculated.

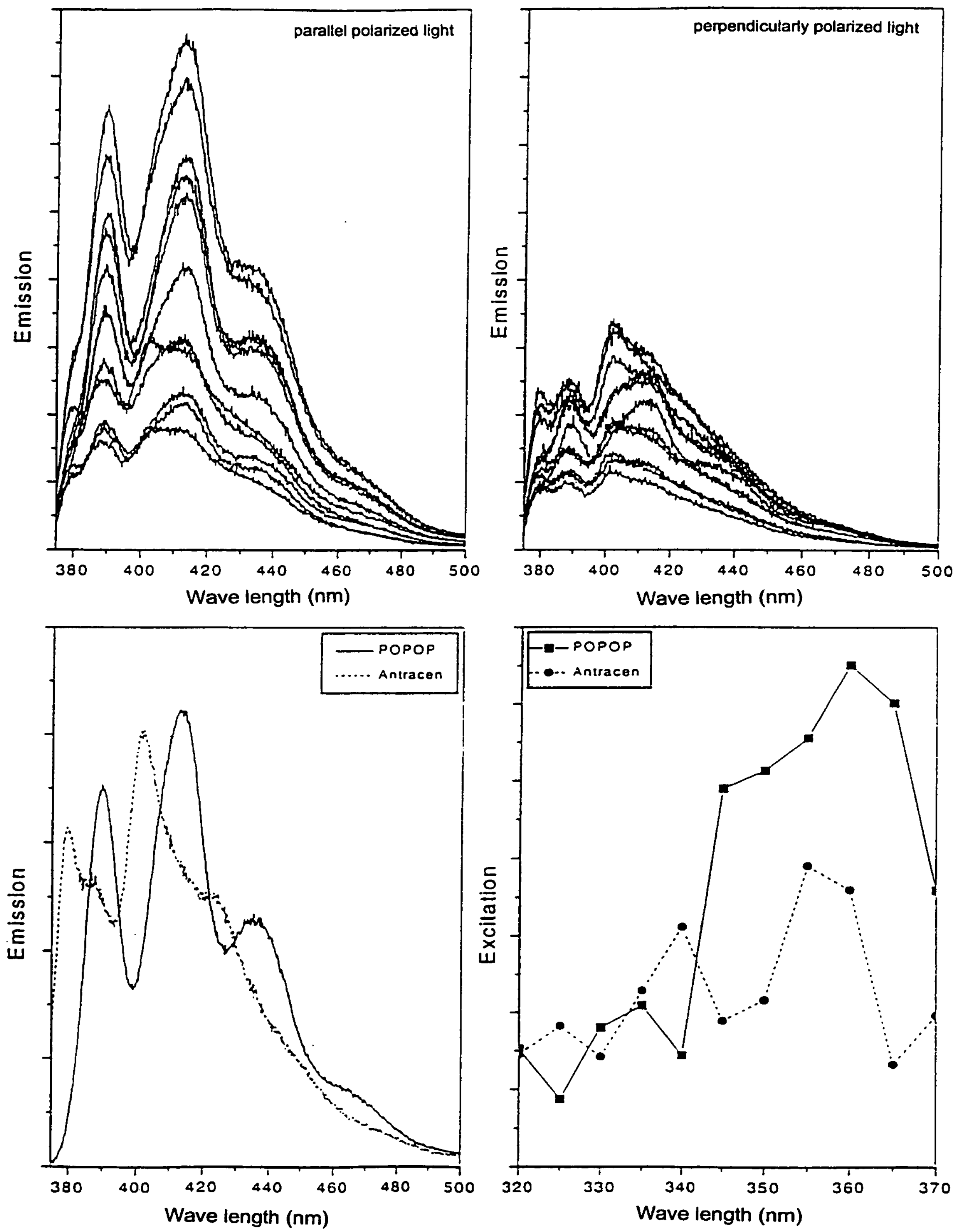
7. A method according to one or more of claims 1-6, wherein more than two data points are monitored in only two of the dimensions.

8. A method according to one or more of claims 1-7, wherein the method generating the multi dimensional response is fluorescence or nuclear magnetic resonance method.

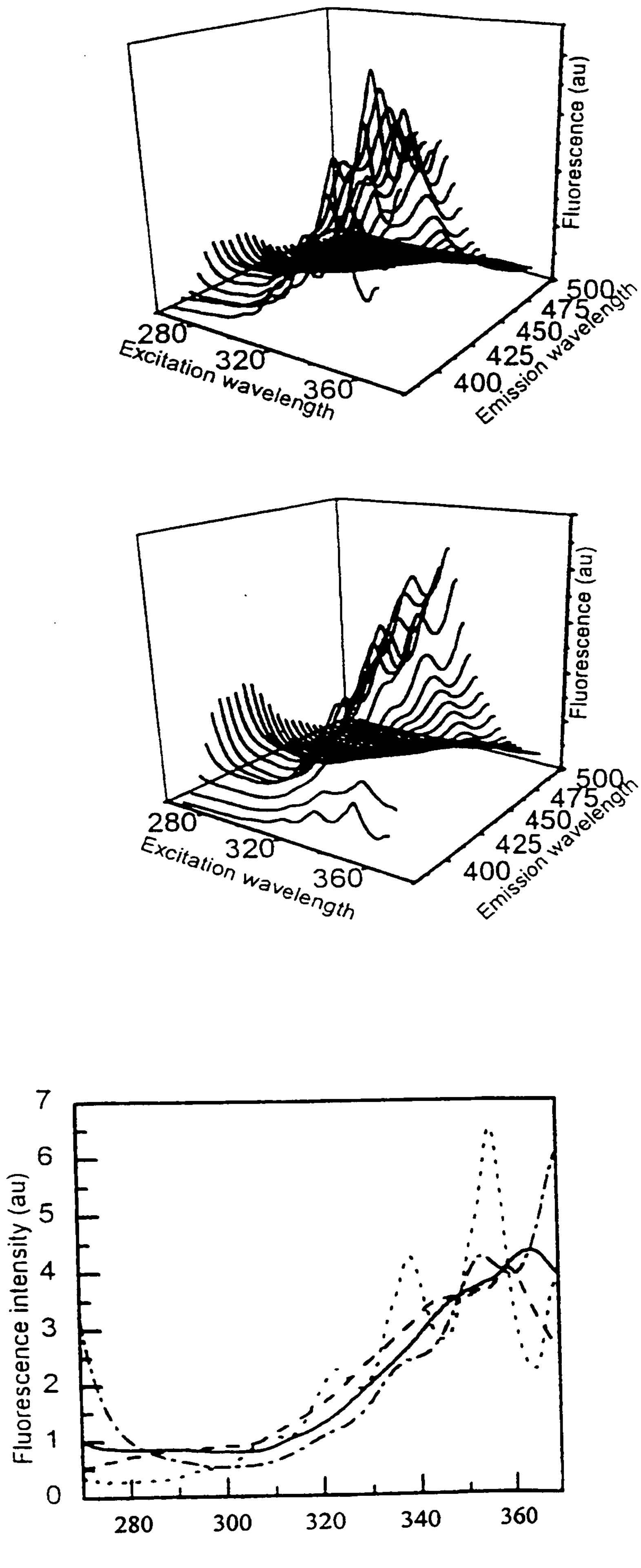
9. A method according to one or more of claims 1-8, wherein the variations along, at least one of the dimensions, is obtained by varying one external parameter, such as time, electrical or magnetical field, temperature, modulation, or polarisation

10. A method according to any of claims 8 or 9, for characterizing a test sample by analysing time dependent emission/excitation spectra, where the time relates to time after excitation, time after the mixing of thge components, time after a certain treatment of the components, such as chromatographic separation or the similar.

11. A method according to any of claims 8 and 9 for characterizing a test sample by analysing two time dependencies, in combination with at least some other dependency, such as the wave length of the light, where the two time dependencies relates to time after excitation, time after the mixing of the components, time after the treatment of the components, such as a chromatographic separation.


parallel polarized light
perpendicularly polarized light
Emission
Wave length (nm)
380 400 420 440 460 480 500
POPOP
Antracen
Excitation
320 330 340 350 360 370

FIGUR 1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5
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3
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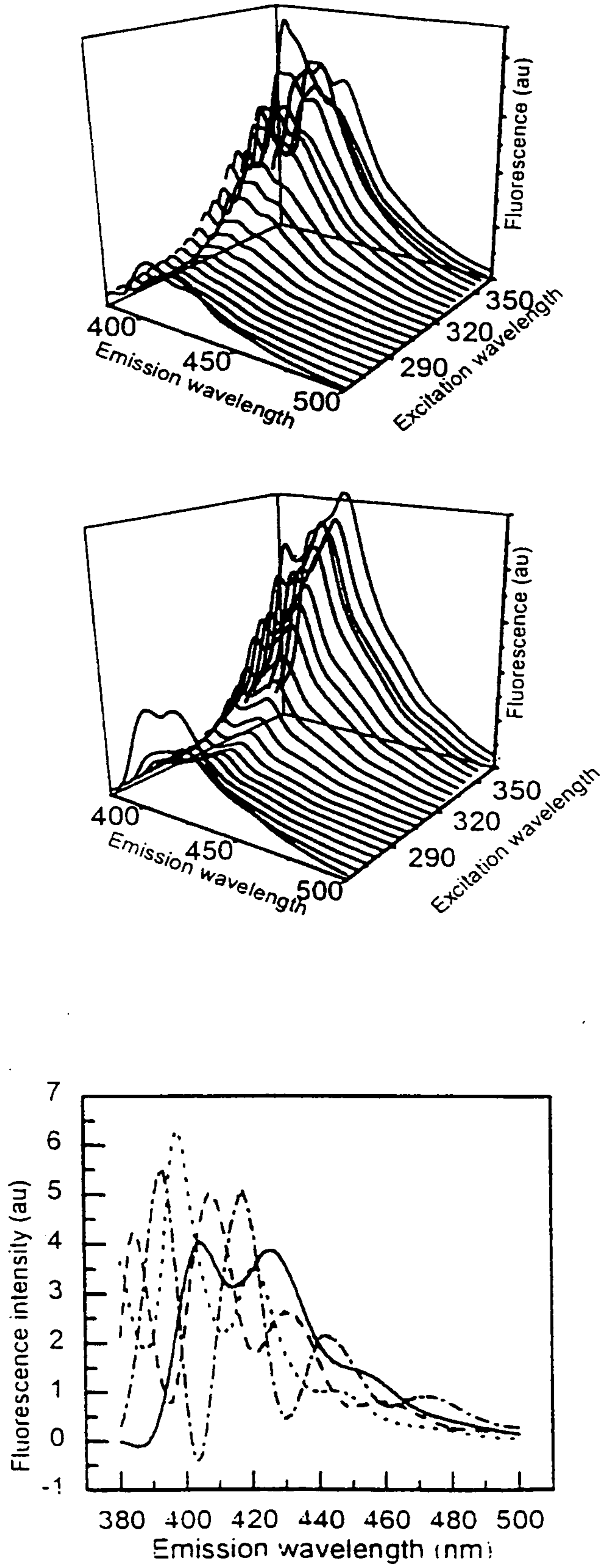
1
0
280
300
320
340
360

FIGUR 2


Fluorescence (au)
400
450
500
Emission wavelength
350
320
290
Excitation wavelength
Fluorescence (au)
400
450
500
Emission wavelength
350
320
290
Excitation wavelength
7
6
5
4
3
2
1
0
-1
Fluorescence intensity (au)
380 400 420 440 460 480 500
Emission wavelength (nm)

FIGUR 3

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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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International Application No.	PCT/SE98/01468
International Filing Date	14-08-1998
Name of receiving Office and "PCT International Application"	The Swedish Patent Office PCT International Application

Applicant's or agent's file reference *(if desired) (12 characters maximum)*	P15558PC

Box No. I TITLE OF INVENTION **Method for characterizing samples**

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State* (i.e. country) of residence if no State of residence is indicated below.)

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Norra Solstensvägen 6 D
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☒ This person is also inventor.

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Teleprinter No.

State *(i.e country)* of nationality: **Sweden**

State *(i.e. country)* of residence: **Sweden**

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State* (i.e. country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

[redacted]T/SE98/01468

14-08-1998

Sheet No. 2

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked):*

Regional Patent

☒ **AP** **ARIPO Patent: GH** Ghana, **GM** Gambia **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA** **Eurasian Patent: AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent: AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT CY

☒ **OA** **OAPI Patent: BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivore, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of PCT *(if other kind of protection or treatment desired, specify on dotted line)* GW

National Patent *(if other kind of protection or treatment desired specify on dotted line)*

- ☒ **AL** Albania
- ☒ **AM** Armenia
- ☒ **AT** Austria
- ☒ **AU** Australia
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria
- ☒ **BR** Brazil
- ☒ **BY** Belarus
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China
- ☒ **CU** Cuba
- ☒ **CZ** Czech Republic
- ☒ **DE** Germany
- ☒ **DK** Denmark
- ☒ **EE** Estonia
- ☒ **ES** Spain
- ☒ **FI** Finland
- ☒ **GB** United Kingdom
- ☒ **GE** Georgia
- ☒ **GH** Ghana
- ☒ **GM** Gambia
- ☒ ~~**GW** Guinea-Bissau~~
- ☒ **HU** Hungary
- ☒ **ID** Indonesia
- ☒ **IL** Israel
- ☒ **IS** Iceland
- ☒ **JP** Japan
- ☒ **KE** Kenya
- ☒ **KG** Kyrgyzstan
- ☒ **KP** Democratic People's Republic of Korea
- ☒ **KR** Republic of Korea
- ☒ **KZ** Kazakstan
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova
- ☒ **MG** Madagascar
- ☒ **MK** The former Yugoslav Republic of Macedonia
- ☒ **MN** Mongolia
- ☒ **MW** Malawi
- ☒ **MX** Mexico
- ☒ **NO** Norway
- ☒ **NZ** New Zealand
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- ☒ **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ ~~Regional = CY Cyprus~~
- ☒ National = HR Croatia
- ☐

In addition to the designations made above, the applicant also makes under Rule 4.9(b) designations which would be permitted under the PCT except the designation(s) of __________.
The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit)*

CANCELLED EX OFFICIO BY IB

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (*in which, or for which, the application was filed*)	Filing Date (*day/month/year*)	Application No.	Office of filing (*only for regional or international application*)
item (1) Sweden	22 April 1998 22/04/98	9801420-2	
item (1)			
item (1)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA/ SE

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (on the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request : 3 sheets
2. description : 15 sheets
3. claims : 3 sheets
4. abstract : 1 sheets
5. drawings : 3 sheets

Total : 25 sheets

This international application is accompanied by the item(s) marked below:

1. ☒ separate signed power of attorney will follow
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) identified in Box No. VI as item(s):
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*

Figure No. 3 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

1998-08-13 Göteborg

Ulf Inger

Göteborgs Patentbyrå

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1. Date of actual receipt of the purported international application: 14-08-1998	2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application.	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority specified by the applicant: ISA/ SE	6. ☐ Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau: 03 SEPTEMBER 1998 (03.09.98)

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Metod för karakterisering av prover

Den föreliggande uppfinningen tillhör kategorin metoder för karakterisering av prover. Dessa används bl.a. för att undersöka testprover från produktion, patienter eller prover som insamlats på annat sätt.

Uppfinningens bakgrund

När ett prov skall karakteriseras med avseende på dess komponenter separeras i allmänhet först komponenterna från varandra för att senare identifieras och mängdbestämmas separat. Det är dock inte alltid möjligt att separera komponenterna åt, eller så är det av tids/kostnadsskäl inte motiverat. Proverna kan då karakterisera spektroskopiskt, varvid komponenterna identifieras genom deras unika spektrala responser.

Om man har en uppsättning prover och vet vilka komponenter de innehåller är det i regel trivialt att bestämma deras koncentrationer spektroskopiskt. Detta gäller även om komponenternas spektrala responser överlappar. Om komponenterna däremot är okända är problemet mycket besvärligare. Situationen analyserades första gången i detalj av matematikerna Lawton och Sylvestre (Technometrics, 13, 1971, 617), som visade att det är omöjligt att finna en unik lösning till och med för ett 2-komponentsystem. 1990 utvecklade vi ett experimentellt förfarande som delvis löste detta problem (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, 1990). Vi visade att om man utförde två spektroskopiska mätningar på varje prov, istället för som tidigare endast en, och mätningarna var sådana att komponenternas bidrag till dessa hade samma intensitetsfördelning, men olika magnitud,

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så kunde både komponenternas spektrala responser och koncentrationer bestämmas. Matematiskt beskrivs dessa mätningarna med ekvationerna:

$$\mathbf{A} = \mathbf{CV} \textit{ eller } a_j(\lambda) = \sum_{i=1}^{r} c_{ij} v_i(\lambda) \qquad j=1,2\ldots n$$

$$\mathbf{B} = \mathbf{CDV} \textit{ eller } b_j(\lambda) = \sum_{i=1}^{r} c_{ij} d_j v_i(\lambda) \qquad j=1,2\ldots n$$

där **A** är en matris som innehåller spektra av det första slaget uppmätta på de n st proverna; **B** är en matris innehållande spektra av det andra slaget uppmätta på samma n prover; **C** är en matris innehållande de r olika komponenternas koncentrationer i de n st proverna, **V** är en matris innehållande komponenternas normaliserade spektra och **D** är en diagonalmatris vars r diagonalelement är kvoterna mellan komponenternas responser i de två mätningarna. Samtliga spektra är digitaliserade i m punkter. Vi visade att endast utifrån informationen i de uppmätta spektra (**A** och **B**) så kunde komponenternas koncentrationer (**C**), deras normaliserade spektrala responser (**V**) samt kvoten mellan deras responser i de två mätningarna (**D**) bestämmas. Vi beskrev också hur antalet komponenter i proverna (r) kunde uppskattas.

En begränsningen med detta tillvägagångssätt är att antalet komponenter inte får överstiga antalet prover, vilket i praktiken innebär att metoden inte är tillämpbar på mindre provserier och kan överhuvudtaget inte appliceras för analys av enstaka prover.

Flera spektroskopiska tekniker, såsom fluorescens, nmr etc., kan generera 2-dimensionella data som beskrivs av ekvationen:

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$$I(\alpha,\beta) = \kappa \sum_{i=1}^{r} I_i(\alpha) c_i I_i(\beta)$$

där signalen, $I(\alpha,\beta)$, mäts som funktion av två variabler, α och β, och är i varje punkt summan av komponenternas bidrag, som är proportionella mot deras koncentrationer (c_i), och produkten av deras (normaliserade) 1-dimentionella responser, $I_i(\alpha)$ och $I_i(\beta)$. Från dessa responser kan komponenterna identifieras. I steady-state fluorescensspektroskopi är $I_i(\alpha)$ och $I_i(\beta)$ komponenternas excitations- och emissionsspektra, och betecknas i regel $I_i^{ex}(\lambda_{ex})$ och $I_i^{em}(\lambda_{em})$, där λ_{ex} och λ_{em} är excitations- och emissionsvåglängderna. Formen hos ett rent ämnes excitationsspektrum är i allmänhet oberoende av den emissionsvåglängd som används vid mätningen, och motsvarande gäller för dess emissionsspektrum. Den uppmätta fluorescenssignalen, om nödvändigt efter korrektion för innerfilter effekten (Kubista et al., The Analyst, 119, 417, 1994), är proportionell mot ämnets koncentration. För ett prov som innehåller flera ämnen är den totala signalen summan av komponenternas bidrag. Eftersom fluorescens mäts i godtyckliga enheter, innehåller eq. 1 en proportionalitetskonstant (κ).

Informationen i det 2-dimensionella spektrumet $I(\alpha,\beta)$ är otillräcklig för att entydigt bestämma komponenternas spektrala responser. Olika approximativa tillvägagångssätt har föreslagits, men dessa fungerar inte fullt tillfredsställande ens för 2-komponentsblandningar (Burdick och Tu, J. Chemometrics, 3, 431, 1989).

Den föreliggande uppfinningen är ett förfarande att analysera ett enstaka testprov, eller par av testprover, utan att använda referenser, på ett sådant sätt att komponenterna kan identifieras.

Beskrivning av figurer

Figur 1. Emissionsspektra uppmätta med olika excitationsvåglängder med parallell polariserat (överst till vänster) respekive med vinkelrätt polariserat (överst till höger) ljus. Nederst till vänster visas komponenternas beräknade emissionsspektra och nederst till höger visas komponenternas beräknade excitationsintensiteter.

Figur. 2. A) Excitationsspektra uppmätta med olika emissionsvåglängder av två lösningar innehållande POPOP, dimetylPOPOP, antracene och difenylantracene. B) Komponenternas beräknade excitationsspektra.

Figur 3. A) Emissionsspektra uppmätta med olika excitationsvåglängder av två lösningar innehållande POPOP, dimetylPOPOP, antracene och difenylantracene. B) Komponenternas beräknade excitationsspektra.

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Kortfattad beskrivning av uppfinningen

Den föreliggande uppfinningen är ett förfarande att analysera testprover på ett sätt att dess komponenter kan identifieras, utan att referensdata behövs. Förfarandet bygger på följande fyra steg:

1. Testprovet analyseras med en metod som genererar en 3-dimentionell respons enligt: $I(\alpha,\beta,\gamma) = \sum_{i=1}^{\bar{r}} \tilde{I}_i(\alpha)\tilde{I}_i(\beta)\tilde{I}_i(\gamma)$, där r är antalet komponenter som bidrar till signalen och $\tilde{I}_i(\alpha)$, $\tilde{I}_i(\beta)$ och $\tilde{I}_i(\gamma)$ är komponenternas arbiträrt normaliserade 1-dimentionella responser, som vanligen utgörs av spektrala eller koncentrationsvariationer.
2. Antalet komponenter, r, som proverna innehåller uppskattas.
3. För varje komponent bestäms dess 1-dimentionella responser $I_i(\alpha)$, $I_i(\beta)$ och $I_i(\gamma)$.
4. Från responserna identifieras komponenterna.

Detaljerad beskrivning av uppfinningen

Som rubriken antyder är den föreliggande uppfinningen ett förfarande att karakterisera ett enstaka testprov på ett sätt som gör det möjligt att identifiera dess komponenterna utan att behöva använda referensprover. Detta sker genom ett strategiskt upplägg av experimentet, som gör det möjligt att registrera en 3-dimentionell respons som är proportionell mot komponenternas koncentrationer, och bidraget från varje komponent är produkten av dess specifika 1-dimentionella responser:

$$I(\alpha,\beta,\gamma) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

Sådan mätning kan t.ex. utföras med vissa former av fluorescensspektroskopi, t.ex. genom tidsupplöst mätning av emissions/excitationsspektra, dvs signalen registreras som funktion av excitationsvåglängd, emissionsvåglängd och tid:

$$I(\lambda_{ex},\lambda_{em},t)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(t)$$

I dessa fall är det ofta lämpligt att sammanföra komponentkoncentrationerna c_i och tidsavklingningarna till en tidsberoende koncentration:

$$I(\lambda_{ex},\lambda_{em},t)=\sum_{i=1}^{r} c_i(t) I_i(\lambda_{ex}) I_i(\lambda_{em})$$

Tiden kan vara tid efter ljuspuls (varvid $c_i(t)$ är proportionellt mot fluorescensavklingningen), tid efter blandning i t.ex. stop-flow experiment (varvid $c_i(t)$ är variationen hos komponent i:s koncentration med tiden), tid efter behandling, såsom fotobleaching (selektiv destruktion av vissa komponenter med ljus), kromatografisk eller annan form av separation, etc. Vid analys av dylika data beräknas komponenternas koncentrationsvariationer samt deras excitations- och emissionsspektra. Det är intressant att notera att intermediära komponenter, som varken är närvarande i början ($c_i(0)=0$) eller i slutet ($c_i(\infty)=0$) av experimentet, kan identifieras utifrån dess beräknade spektra.

Det är också möjligt att variera ljusets polarisation:

$$I(\lambda_{ex},\lambda_{em},\alpha)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

eller, om fasmodulerat ljus används, modulationens frekvens:

$$I(\lambda_{ex},\lambda_{em},\nu)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\nu)$$

osv.

Det är också möjligt att variera yttre parametrar som påverkar komponenterna koncentrationer, såsom temperatur (tryck, volym, etc):

$$I(\lambda_{ex},\lambda_{em},T)=\sum_{i=1}^{r} c_i(T)I_i(\lambda_{ex})I_i(\lambda_{em})$$

eller yttre parametrar som påverkar intensiteten i komponenternas responser, såsom externa magnetfält (elektriska fält, etc)

$$I(\lambda_{ex},\lambda_{em},M)=\sum_{i=1}^{r} c_i I_i(\lambda_{ex})I_i(\lambda_{em})I_i(M).$$

Den spektroskopiska tekniken behöver naturligtvis inte vara fluorescens. Förfarandet kan utföras med de flesta tekniker som genererar 3-dimensionella responser, t.ex. kärnmagnetisk resonans (nmr), masspektrometri etc. Den kan även utföras med de flesta tekniker som genererar en 2-dimensionell responser om komponenternas responser påverkas externa parametrar. Slutligen kan förfarandet även användas med en teknik som genererar 1-dimensionell respons, men då krävs att man samtidigt varierar två olika externa parametrar och att dessas inverkan på komponenternas responser är oberoende så att deras bidrag kan faktoriseras.

Uppfinningen kräver att åtminstone två data punkter bestäms i var och en av de 3 dimensionerna, dvs:

$I_i(\alpha)$ där $\alpha_1,\alpha_2...\alpha_n$ $1 \geq 2$

$I_i(\beta)$ där $\beta_1,\beta_2...\beta_m$ $m \geq 2$

$I_i(\gamma)$ där $\gamma_1,\gamma_2...\gamma_l$ $n \geq 2$

Att enbart bestämma två datapunkter i samtliga dimensioner är dock sällan meningsfullt, eftersom noggrannheten i de beräknade responserna är då i regel otillräcklig för att komponenterna ska kunna identifieras. Däremot går det utmärkt att endast ha två datapunkter i en av dimensionerna, t.ex. l=2 (och m>>2 och n>>2). Det har dessutom

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fördelen att den numeriska behandlingen av data underlättas, eftersom komponenternas responser kan beräknas med snabba algoritmer såsom Procrustes rotation och GRAM (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, 1990; Wilson, Sanches & Kowalski, J. Chemometrics, 3 493, 1989). I det generella fallet när samtliga l, n och m är större än 2, är lösningsförfarandet mer komplicerat och därmed betydligt mer tidskrävande (Liwo et al., Computers Chem. 89-96, 21, 1997). Dessutom, är det ofta aktuellt att utföra experiment så att endera utav m och n är betydligt större än den andra. Skälet är att det i regel är tillräckligt för komponenternas identifiering, att med hög noggrannhet bestämma en utav deras 1-dimensionella responser. Detta är t.ex. fallet i exempel 1, där endast komponenternas emissionsresponser bestämds med hög noggrannhet.

Uppfinningen är inte begränsad till mätningar som genererar 3-dimensionell respons, utan även högre ordningens responser kan användas. Generellt gäller att responsen skall vara linjär och bidraget från varje komponent skall vara produkten av dess 1-dimensionella responser:

$$I(\alpha,\beta,\gamma,\delta....) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta)....$$

Naturligtvis, ju högre dimensionen är ju mer tidskrävande blir den numeriska behandlingen av mätdata. Dock, med tanke på den mycket snabba utveckling som sker inom dataområdet, kommer detta knappast vara en praktisk begränsning i framtiden.

Proverna som analyseras skall innehålla mestadels samma komponenter, och dessa ska förekomma i olika relativa koncentrationer. Proverna analyseras parvis med en 2-dimentionell metod som ger en respons som

är proportionell mot komponenternas koncentrationer och produkten av deras 1-dimentionella responser. Detta kan uttryckas:

$$I^A(\alpha,\beta)=\sum_{i=1}^{r} I_i(\alpha)c_i^A I_i(\beta)$$

$$I^B(\alpha,\beta)=\sum_{i=1}^{r} I_i(\alpha)c_i^B I_i(\beta)$$

där $I^A(\alpha,\beta)$ och $I^B(\alpha,\beta)$ är spektra på de två proverna, som i fortsättningen betecknas A och B, uppmätta som funktion av variablerna α och β, r är det totala antalet komponenter som bidrar till spektra, $I_i(\alpha)$ och $I_i(\beta)$ är komponenternas normaliserade 1-dimentionella responser, och c_i^A och c_i^B är deras koncentrationer. I steady-state fluorescensspektroskopi är $I_i(\alpha)$ komponenternas normaliserade excitationsspektra, $I_i^{ex}(\lambda_{ex})$, och $I_i(\beta)$ är deras normaliserade emissionsspektra, $I_i^{em}(\lambda_{em})$:

$$I^A(\lambda_{ex},\lambda_{em})=\sum_{i=1}^{r} I_i^{ex}(\lambda_{ex})c_i^A I_i^{em}(\lambda_{em})$$

$$I^B(\lambda_{ex},\lambda_{em})=\sum_{i=1}^{r} I_i^{ex}(\lambda_{ex})c_i^B I_i^{em}(\lambda_{em})$$

Informationen i dessa spektra behandlas i två steg. Först bestäms antalet komponenter, r, och sedan komponenternas 1-dimensionella responser, $I_i^{ex}(\lambda_{ex})$ och $I_i^{em}(\lambda_{em})$.

När r bestämds beräknas komponenternas spektrala responser. Ekvationerna:

$$I^A(\lambda_{ex},\lambda_{em})=\sum_{i=1}^{r} I_i^{ex}(\lambda_{ex})c_i^A I_i^{em}(\lambda_{em})$$

$$I^B(\lambda_{ex},\lambda_{em})=\sum_{i=1}^{r} I_i^{ex}(\lambda_{ex})c_i^B I_i^{em}(\lambda_{em})$$

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kan i matrisform skrivas:

$$\mathbf{A}=\mathbf{X}\mathbf{C}^{\mathbf{A}}\mathbf{M}$$
$$\mathbf{B}=\mathbf{X}\mathbf{C}^{\mathbf{B}}\mathbf{M}$$

Där **A** och **B** är matriser innehållande de uppmätta spektra, **X** är en matris innehållande komponenternas normaliserade excitationsspektra, **M** är en matris innehållande deras normaliserade emissionsspektra, och $\mathbf{C}^{\mathbf{A}}$ och $\mathbf{C}^{\mathbf{B}}$ är diagonalmatriser innehållande komponenternas koncentrationer. Genom att omnormalisera endera **X** eller **M**, kan ekvationssystemet omskrivas till:

$$\mathbf{A}=\mathbf{X}\mathbf{M}$$
$$\mathbf{B}=\mathbf{X}\mathbf{D}\mathbf{M}$$

Där **D** är en diagonalmatris innehållande kvoterna mellan komponenternas koncentrationer ($\mathbf{D}=\mathbf{C}^{\mathbf{B}}/\mathbf{C}^{\mathbf{A}}$). Utifrån **A** och **B** kan **X**, **M** samt **D** beräknas med kända metoder såsom t.ex. Procrustes rotation (Kubista, Chemometrics and Intelligent Laboratory Systems, 7, 273, 1990) och GRAM (Wilson, Sanches & Kowalski, J. Chemometrics, 3 493, 1989).

Sammanfattningsvis utgörs den föreliggande uppfinningen av ett förfarande att experimentellt studera två prover spektroskopiskt så att den information som finns i de experimentella spektra är tillräcklig för att bestämma antalet komponenter i proverna (r), deras spektrala responser i den 1:a dimensionen, $I_i(\alpha)$, deras spektrala responser i den

2:a dimensionen, $I_i(\beta)$, samt kvoterna mellan deras koncentrationer (c_i^B/c_i^A).

Den mest uppenbara användning av uppfinningen är för analys av två prover som innehåller gemensamma komponenter. Alla komponenter behöver inte vara gemensamma, men majoriteten av dem som bidrar spektroskopiskt bör vara det (Booksh & Kowalski, J. Chemotrics, 8, 287, 1994). Komponenternas antal är godtyckligt, och kan överstiga 2.

En annan användning av uppfinningen är att karakterisera enstaka prov genom att först dela upp det i två delprover som innehåller de ingående komponenterna i olika proportioner. Detta kan åstadkommas på ett flertal sätt, t.ex. genom att provet filtreras, extraheras, kromatograferas, dialyseras, centrifugeras, utfälls, delas upp med elektriskt fält, etc. Alternativt kan ursprungsprovet användas som ett prov och en delmängd av detta, som skapas så att komponenterna förekommer i andra proportioner, används som det andra provet. Denna delmängd kan åstadkommas genom att selektivt avlägsna vissa komponenter t.ex. medelst adsorption, utfällning, nedfrysning, destillation, selektiv degradering (t.ex. med ljus, värme, radiolys), etc. Ytterligare en möjlighet att skapa två prover från ett är att ändra betingelserna för mätningen, t.ex. genom att ändra temperatur, tryck etc. Separationsmetoder, såsom olika former av kromatografi, är intressanta, eftersom komponenterna separeras rumsligt och ett, i princip, godtyckligt antal prover kan erhållas som kan analyseras parvis. Med spektroskopiska tekniker som snabbt genererar 2-dimensionella spektra kan dessutom detektionen ske on-line.

Ytterligare en användning av uppfinningen är att med hög noggrannhet bestämma koncentrationerna av komponenterna i ett testprov relativt ett standardprov. Standardprovet och testprovet analyseras som par, och kvoten mellan komponenternas koncentrationer erhålls som diagonalelementen i **D** matrisen.

Av särskilt intresse är 2-dimensionella spektra där en av dimensionerna är tid, avseende tid efter störning, såsom relaxationstid. I dag finns, t.ex., fluorescensinstrument med vilka man kan mäta kompletta spektra som funktion av tid efter belysning (antingen direkt efter belysning med ljuspuls, eller indirekt med fasmodulationsteknik). Detta ger med α som tid och β som våglängd ekvationssystemet:

$$I^A(t,\lambda) = \sum_{i=1}^{r} I_i(t) c_i^A I_i(\lambda)$$

$$I^B(t,\lambda) = \sum_{i=1}^{r} I_i(t) c_i^B I_i(\lambda)$$

från vilket r, $I_i(t)$, $I_i(\lambda)$ och (c_i^B/c_i^A) kan bestämmas.

Exempel

Uppfinningen illustreras med fyra exempel

Exempel 1

Ett prov karakteriseras med fluorescensspektroskopi där excitationsvåglängd, emissionsvåglängd och ljusets polarisation varieras (Figur 1). Detta ger upphov till ett 3-dimensionellt spektrum enligt:

$$I(\lambda_{ex},\lambda_{em},\alpha) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha)$$

I exemplet används 650 olika emissionsvåglängder (m), 11 olika excitationsvåglängder (n) och 2 olika polarisationer ($\alpha = 0°$ benämnt parallell polarisation och $\alpha = 90°$ benämnt vinkelrätt polarisation, l). Från den uppmätta responsen, $I(\lambda_{ex}, \lambda_{em}, \alpha)$, uppskattades först antalet komponenter (r) till 2 (med statiskt test och visuell inspektion av principalkomponenterna). Sedan beräknades de komponentspecifika responserna. För detta utnyttjade man det faktum att endast två datapunkter registrerats i en av dimensionerna (polarisation), och skrev om den 3-dimensionella responsen till 2 st 2-dimensionella responser:

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 0°) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 0°)$$

$$I(\lambda_{ex}, \lambda_{em}, \alpha = 90°) = \sum_{i=1}^{r} c_i I_i(\lambda_{ex}) I_i(\lambda_{em}) I_i(\alpha = 90°)$$

Dessa kan beskrivas med ekvationssystemet:

$\mathbf{I}^0 = \mathbf{X}\alpha^0\mathbf{M}$

$\mathbf{I}^{90} = \mathbf{X}\alpha^{90}\mathbf{M}$

som kan lösas med Procrustes rotation (Kubista, Chemometrics and Intelligent Laboratory Systems 7, 273, 1990). Detta gav komponenternas normaliserade excitationsintensiteter som matris **X** ($\tilde{I}_i(\lambda_{ex})$, visas nederst till höger i figur 1), komponenternas normaliserade emissionsintensiteter som matris **M** ($\tilde{I}_i(\lambda_{em})$, visas nederst till vänster i figur 1), samt kvoterna mellan komponenterna responser av ljus med olika polarisation $\left(\tilde{I}_1(\alpha = 90°)/\tilde{I}_1(\alpha = 0°)\right) = 0.25$ och $\left(\tilde{I}_2(\alpha = 90°)/\tilde{I}_2(\alpha = 0°)\right) = 0.94$). Från de beräknade komponentspecifika responserna, särskilt emissionsspektra, kunde komponenterna identifieras som p-bis[2-(5-phenyloxazolyl)] benzen (POPOP) och antracen. Slutligen , genom att jämföra med standardspektra av POPOP och antracen, kunde koncentrationerna uppskattas till någon mikromolar.

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Exempel 2

Två lösningar innehållande färgämnena POPOP, dimetylPOPOP, antracene och difenylantracene i olika proportioner tillreddes. På dessa mättes fluorescensexcitationsspektra vid ett flertal emissionsvåglängder. Komponenternas antal bestämdes till 4 med statiska test, och komponenternas excitationsspektra (Figur 1), emissionsintensiteter samt förhållande mellan deras koncentrationer i de två proverna beräknades.

Exempel 3

På samma lösningar som i exempel 1 mättes fluorescens emissionsspektra vid ett flertal excitationsvåglängder. Komponenternas antal bestämdes till 4 med statiska test, och komponenternas emissionsspektra (Figur 2), excitationsintensiteter, samt förhållande mellan deras koncentrationer i de två proverna beräknades.

Exempel 4

Karakterisering av prover innehållande färgämnet tiazolorange och polymeren poly(dG). Proverna analyseras parvis med 2-dimensionell fluorescensspektroskopi . De innehåller tiazolorange och poly(dG) i förhållandena [tiazolorange]/[poly(dG)] 0.05 och 0.025. Varken poly(dG) eller färgämnet är fluorescenta i sig själva, utan fluorescensen uppkommer när tiazolorange binder till polymeren. Proverna analyserades på två oberoende sätt. I en analys mättes fluorescensexcitationsspektra med olika emissionsvåglängder. Antalet fluorescenta compo-

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nenter identifierades till två med statiska tester, och deras excitationsspektra och emissionsintensiteter beräknades. I den andra analysen mättes fluorescensemissionsspektra med ett antal olika excitationsvåglängder. Åter identifierades antalet komponenter till två, och deras emissionsspektra och excitationsintensiteter beräknades.

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Patentkrav

1. Ett förfarande att karakterisera testprover som kännetecknas därav att:
   a) ett prov, eller par av prover, karakteriseras med mätteknik så att en multidimensionell respons genereras enligt:
   $$I(\alpha,\beta,\gamma,\delta....) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma) I_i(\delta)....,$$
   b) den uppmätta responsen bryts ned till ett ortogonalt basset, t.ex. med principalkomponentuppdelning,
   c) antalet komponenter i provet (r) uppskattas,
   d) komponenternas arbiträrt normaliserade 1-dimensionella, responser beräknas.
2. Ett förfarande enligt krav 1 där proverna är två till antalet och analyseras med en metod som genererar 2-dimentionell respons

   enligt: $I(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i I_i(\beta)$ och komponenternas 1-dimensionella responser och kvoterna mellan deras koncentrationer i de två proverna, c_i^B / c_i^A, beräknas genom att lösa ekvationssystemet
   $$I^A(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^A I_i(\beta)$$
   $$I^B(\alpha,\beta) = \sum_{i=1}^{r} I_i(\alpha) c_i^B I_i(\beta)$$
3. Ett förfarande enligt krav 2 där de två proverna genereras utifrån ett prov.
4. Ett förfarande enligt krav 1 eller 2 där ett av proverna används som standardprov för att bestämma komponenternas koncentrationer i ett testprov.
5. Ett förfarande enligt krav 1 där ett enstaka prov analyseras med en teknik som genererar 3-dimensionell respons:
   $$I(\alpha,\beta,\gamma) = \sum_{i=1}^{r} c_i I_i(\alpha) I_i(\beta) I_i(\gamma)$$

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och komponenternas arbiträrt normaliserade 1-dimensionella responser, $\tilde{I}_i(\alpha)$, $\tilde{I}_i(\beta)$ och $\tilde{I}_i(\gamma)$ beräknas.

6. Ett förfarande enligt krav 1 där ett enstaka prov analyseras med en teknik som genererar 2-dimensionell respons samtidigt som externa parametrar varieras så att komponenternas koncentrationer ändras i tiden:

$$I(\alpha,\beta,\gamma)=\sum_{i=1}^{r} c_i(t)I_i(\alpha)I_i(\beta)$$

och komponenternas arbiträrt normaliserade 1-dimensionella responser, $\tilde{I}_i(\alpha)$, $\tilde{I}_i(\beta)$ och deras koncentrationsförändringar $c_i(t)$ beräknas.

7. Ett förfarande enligt något av kraven 1 till 6 där mer än två datapunkter registreras i endast två av dimensionerna.
8. Ett förfarande enligt någon av kraven 1 till 7 där metoden som genererar den multidimensionell responsen är fluorescens eller kärnmagnetisk resonans.
9. Ett förfarande enligt något av kraven 1 till 8 där variationerna utmed, åtminstone en av dimensionerna, åstadkoms genom att variera en extern parameter, såsom tid, elektriskt eller magnetiskt fält, temperatur, modulation, polarisation
10. Ett förfarande enligt något av kraven 8 och 9 att karakterisera ett testprov genom att analysera tidsberoende emissions/excitationsspektra, där tiden avser tid efter excitation, tid efter komponenternas blandande, tid som komponenterna utsatts för viss behandling såsom kromatografisk separation och liknande.
11. Ett förfarande enligt något av kraven 8 och 9 att karakterisera ett testprov genom att analysera två tidsberoenden, i kombination med åtminstone något annat beroende, såsom ljusets våglängd, där de två

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tidsberoenden avser två utav tid efter excitation, tid efter komponenternas blandande, tid som komponenterna utsatts för behandling såsom kromatografisk separation.

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Sammanfattning

Uppfinningen är ett förfarande att karakterisera enstaka testprover med tekniker som genererar flerdimensionella responser från vilka provets komponenter kan identifieras. Förfarandet kräver inga referenser och är tillämpbart även på prover som är färre till antalet än det antal komponenter som de innehåller.

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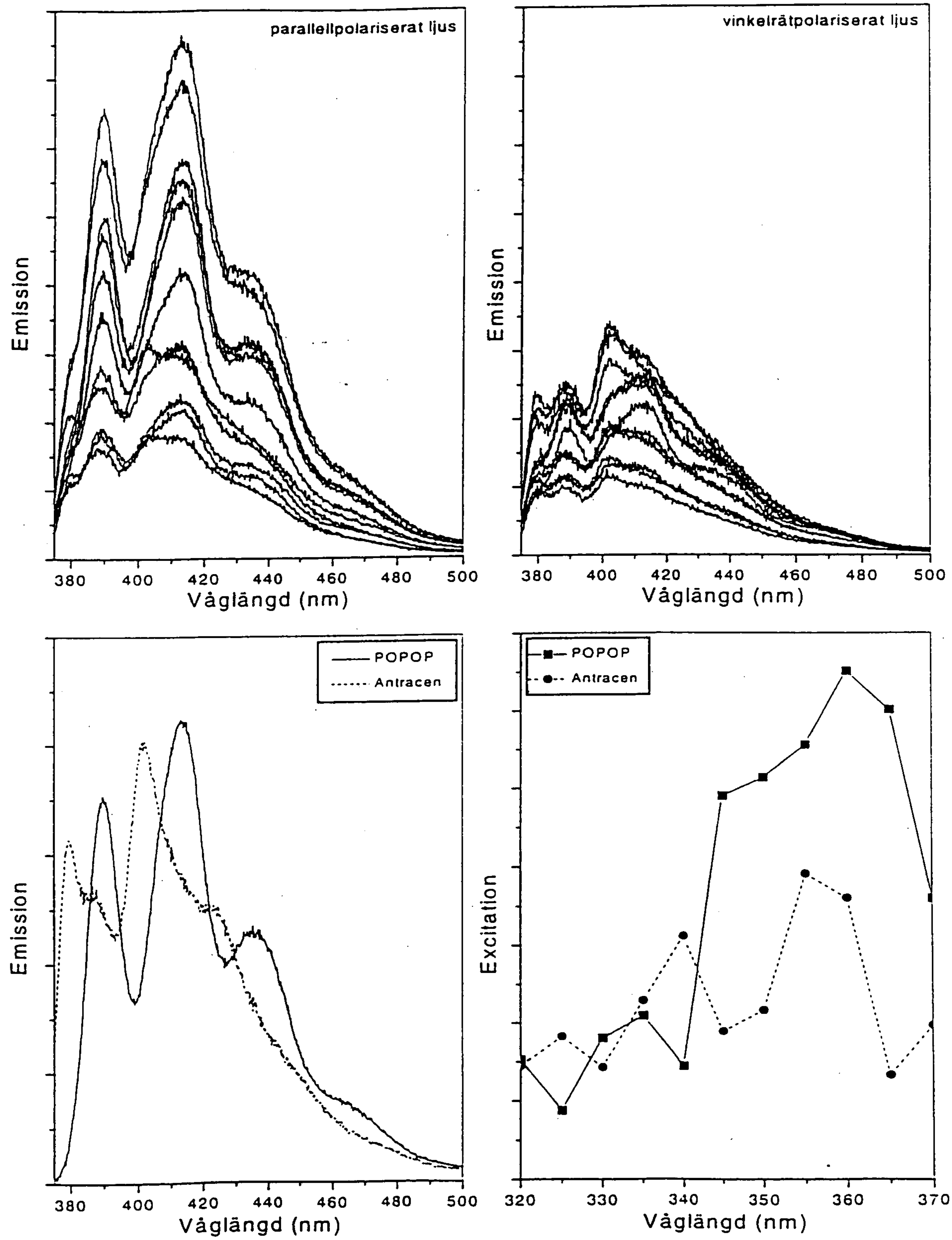

FIGUR 1

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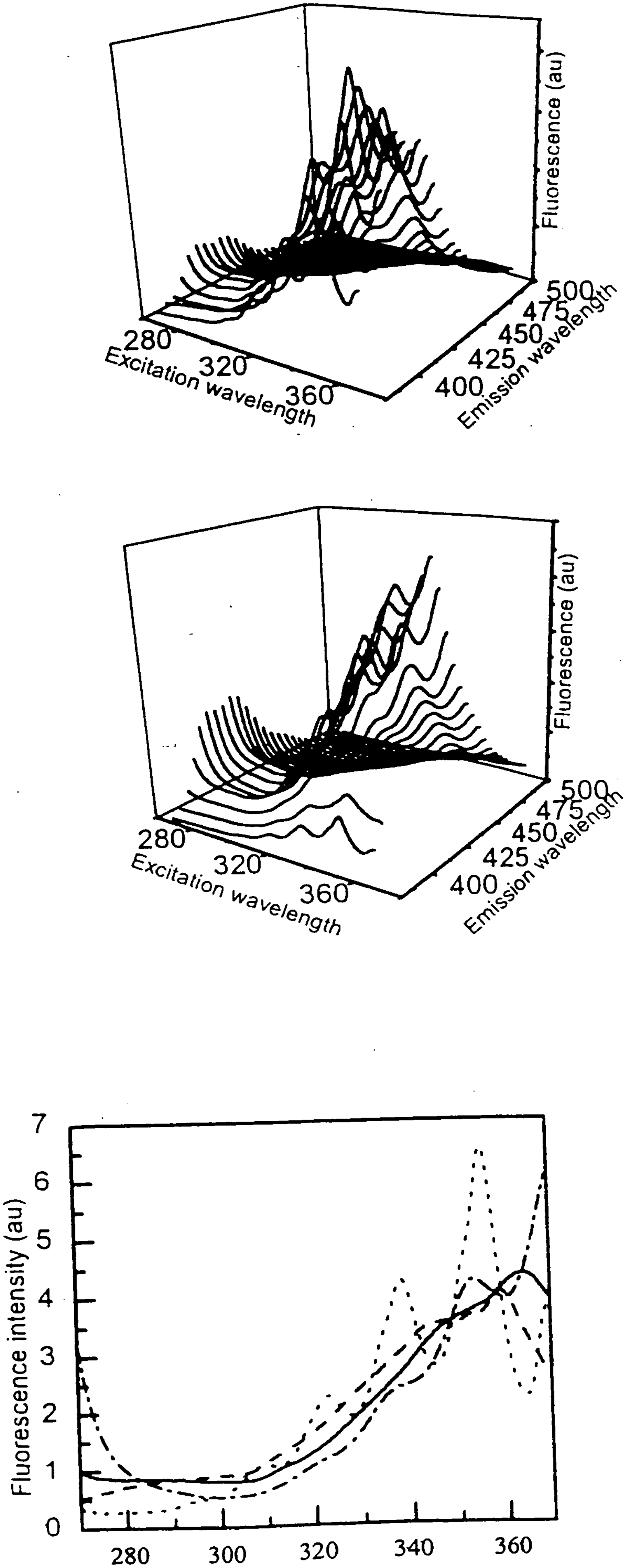

FIGUR 2

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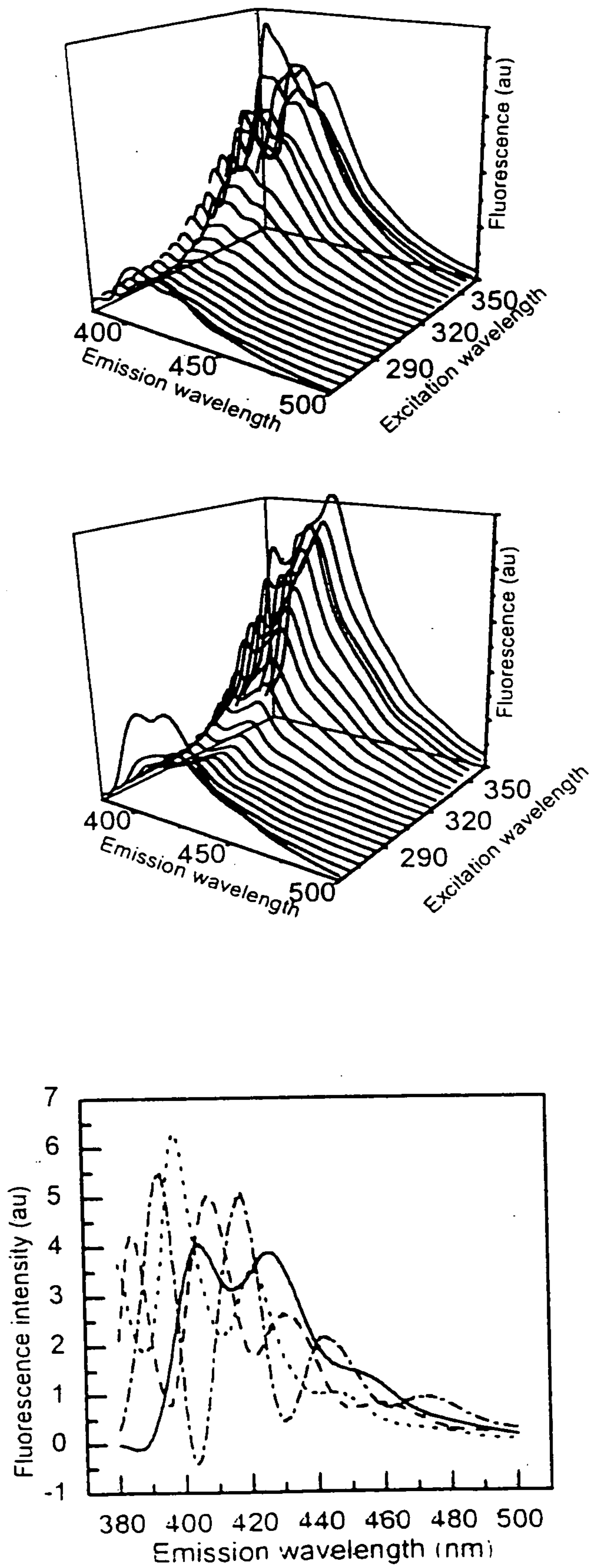

FIGUR 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01468

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 21/64, G01N 24/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5498875 A (ROBERT J. OBREMSKI ET AL), 12 March 1996 (12.03.96), column 2, line 44 - line 46; column 6, line 6 - line 12; column 9, line 9 - line 57, column 11, line 3 - line 42 --	1-11
A	WO 9531713 A1 (EKA NOBEL AB), 23 November 1995 (23.11.95), page 14, 19-23 --	1-11
A	Journal of Chemometrics, Volume 3, 1989, Bruce E. Wilson et al, "AN IMPROVED ALGORITHM FOR THE GENERALIZED RANK ANNIHILATION METHOD", page 493 - page 498, whole document --	1-11

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 1998	18 -11- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Ulf Nyström Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01468

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemometrics and Intelligent Laboratory Systems, Volume 7, 1990, Mikael Kubista, "A New Method for the Analysis of Correlated Data Using Procrustes Rotation which is Suitable for Spectral Analysis", page 273 - page 279, whole document	1-11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

03/11/98

International application No.

PCT/SE 98/01468

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5498875 A	12/03/96	CA 2173981 A	22/02/96
		EP 0723657 A	31/07/96
		JP 9507579 T	29/07/97
		WO 9605500 A	22/02/96
WO 9531713 A1	23/11/95	AT 161631 T	15/01/98
		AT 165446 T	15/05/98
		AU 2582395 A	05/12/95
		AU 2582495 A	05/12/95
		CA 2189857 A	23/11/95
		CA 2189858 A	23/11/95
		DE 69501333 D,T	16/04/98
		DE 69502189 D,T	03/09/98
		EP 0759160 A,B	26/02/97
		SE 0759160 T3	
		EP 0760094 A,B	05/03/97
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		FI 960243 A	17/01/97
		FI 960244 A	17/01/97
		JP 10500215 T	06/01/98
		JP 10500216 T	06/01/98
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		NO 964851 D	00/00/00
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		US 5680321 A	21/10/97
		WO 9531714 A	23/11/95

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

GÖTEBORGS PATENTBYRÅ DAHLS AB
Sjöporten 4
S-417 64 Göteborg
SUÈDE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 23 February 2000 (23.02.00)	
Applicant's or agent's file reference P15558PC	**IMPORTANT NOTIFICATION**
International application No. PCT/SE98/01468	**International filing date** (day/month/year) 14 August 1998 (14.08.98)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
GÖTEBORGS PATENTBYRÅ Sjöporten 4 S-417 64 Göteborg Sweden	Telephone No. +46 31 507700 Facsimile No. +46 31 7790640 Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
GÖTEBORGS PATENTBYRÅ DAHLS AB Sjöporten 4 S-417 64 Göteborg Sweden	Telephone No. +46 31 507700 Facsimile No. +46 31 7790640 Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☒ the International Preliminary Examining Authority
☐ the designated Offices concerned
☒ the elected Offices concerned
☐ other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer F. Gateau Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

GÖTEBORGS PATENTBYRÅ DAHLS AB
Sjöporten 4
S-417 64 Göteborg
SUÈDE

Date of mailing (day/month/year) 23 February 2000 (23.02.00)	
Applicant's or agent's file reference P15558PC	**IMPORTANT NOTIFICATION**
International application No. PCT/SE98/01468	**International filing date** (day/month/year) 14 August 1998 (14.08.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
KUBISTA, Mikael Norra Solstensvägen 6 D S-435 31 Mölnlycke Sweden	SE	SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
KUBISTA, Mikael Nedre Solstensv. 6 D S-435 31 Mölnlycke Sweden	SE	SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer F. Gateau Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 February 2000 (23.02.00)	
International application No. PCT/SE98/01468	**Applicant's or agent's file reference** P15558PC
International filing date (day/month/year) 14 August 1998 (14.08.98)	**Priority date** (day/month/year) 22 April 1998 (22.04.98)
Applicant KUBISTA, Mikael	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

11 November 1999 (11.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer F. Gateau Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

REC'D 2 1 MAR 2000
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference p15558pc00/ca	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE98/01468	International filing date *(day/month/year)* 14.08.1998	Priority date *(day/month/year)* 22.04.1998
International Patent Classification (IPC) or national classification and IPC7 G01N 21/64, G01N 24/08		
Applicant KUBISTA, Mikael		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of ______ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.11.1999	09.03.2000
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Jonas Andersson /itw Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/01468

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☒ the international application as originally filed.

☐ the description, pages ____________, as originally filed,
pages ____________, filed with the demand,
pages ____________, filed with the letter of ____________,
pages ____________, filed with the letter of ____________.

☐ the claims, Nos. ____________, as originally filed,
Nos. ____________, as amended under Article 19,
Nos. ____________, filed with the demand,
Nos. ____________, filed with the letter of ____________,
Nos. ____________, filed with the letter of ____________.

☐ the drawings, ~~sheets~~/fig ____________, as originally filed,
~~sheets~~/fig ____________, filed with the demand
~~sheets~~/fig ____________, filed with the letter of ____________,
~~sheets~~/fig ____________, filed with the letter of ____________.

2. The amendments have resulted in the cancellation of:

☐ the description, pages ____________

☐ the claims, Nos. ____________

☐ the drawings, ~~sheets~~/fig ____________

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-11	YES
	Claims		NO
Inventive step (IS)	Claims	1-11	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations

The invention relates to a method for spectroscopic (e.g. fluorescence or nuclear magnetic resonance) characterization of a single sample using a monitoring technique that generates a multi-dimensional response proportional to the concentration of different components in the sample. The response monitored is broken down to an orthogonal base, whereafter the number of components in the sample is estimated and arbitrary normalized 1-dimensional responses of the components are calculated.

The invention also includes the idea of varying an external parameter, such as time, electric or magnetic field, temperature, modulation or polarisation in order to accomplish variations along at least one of the dimensions.

In prior art methods, which could be represented by the cited article "A New Method for the Analysis of Correlated Data Using Procrustes Rotation which is Suitable for Spectral Analysis", Chemometrics and Intelligent Laboratory Systems, 7 (1990), the number of components are not allowed to exceed the number of samples. From a practical point of view this method can not be utilized on small sample series and can not be applied to the analyzation of isolated samples.

The claimed method does not require any references and is applicable even on samples that are fewer than the number of components they contain and also when the number of components are unknown.

Therefore, the invention according to claims 1-11 is novel, is considered to involve an inventive step and to be industrially applicable.